Hyperhomocysteinemia, nutritional status, and cardiovascular disease in hemodialysis patients

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Background. Hyperhomocysteinemia, cardiovascular disease (CVD), and malnutrition are common in patients with end-stage renal disease (ESRD). This study was designed to assess possible relationships between total plasma homocysteine (tHcy), nutritional status, and ischemic CVD.

Methods. We performed a cross-sectional study in 117 unselected patients on maintenance hemodialysis (HD) treatment, among whom there was a high prevalence of malnutrition (56%), as assessed by the subjective global nutritional assessment (SGNA), and a high prevalence of CVD (60%), and prospectively, we followed-up the overall mortality for four years.

Results. The level of tHcy was elevated in 95% of the HD patients, and that of total plasma cysteine (tCys) was also significantly elevated, while the plasma concentrations of methionine (Met), serine (Ser), and taurine (Tau) were significantly lower than those in healthy controls. The 65 patients who were malnourished according to the SGNA score had significantly lower levels of serum albumin (S_{Alb}), plasma IFG-1 (p-IGF-1), tHcy, tCys, and Met than the 52 patients with normal nutritional status, whereas the levels of Ser, Tau, plasma folate, and vitamin B_{12} were similar in the two groups. The prevalence of malnutrition was 30% in the 47 patients without CVD and was significantly higher (70%, $P < 0.001$) in the 70 patients with CVD, who also had lower tHcy, S_{Alb}, plasma IGF-1, serum creatinine (S_{Cr}), and blood hemoglobin. The tHcy levels were positively correlated with S_{Alb}, Met, tCys, and S_{Cr}. Stepwise, multiple-regression analysis showed that tCys, S_{Alb}, and normalized protein equivalent of nitrogen appearance (nPNA), an indicator of protein intake, were independent predictors of tHcy. The patients with tHcy <24 μmol/L (median value) had a significantly worse four-year survival than those with a higher tHcy (≥ 24 μmol/L).

Conclusions. Our results demonstrate that most of HD pa-

tients have grossly elevated tHcy levels, but that the absolute level appears to be dependent on nutritional status, protein intake, and S_{Alb}. The results also suggest that the lower tHcy levels in patients with CVD than in those without CVD may be related to the higher prevalence of malnutrition and hypoalbuminemia in the CVD patients. This is also in accordance with our observation that the patients with lower tHcy had a worse survival rate than those with higher tHcy, considering that malnutrition is a strong risk factor for mortality and that CVD is the most common cause of death in ESRD patients.

Hyperhomocysteinemia is considered an independent risk factor for atherosclerosis in the general population [1, 2]. However, the relationship between hyperhomocysteinemia and vascular events is complex and not well understood, and it is not clear why a high level of plasma total homocysteine (tHcy) appears to promote atherosclerosis. Experimental studies suggest that homocysteine may enhance lipoprotein oxidation, increases smooth muscle cell proliferation, induces endothelial dysfunction, induces endothelial activation of factor V, and reduces protein C activation by arterial and venous endothelial cells [3].

The risk of premature and progressive occlusive vascular disease is high in chronic uremic patients and accounts for more than 40% of the deaths in dialysis patients [4]. The mechanism is unclear, although hypertension, disorders of lipid metabolism, glucose intolerance, smoking, and anemia, as well as inflammation/infection, may be relevant [4, 5]. In addition, malnutrition in uremic patients may be a predisposing factor to cardiac disease or may contribute to a poor prognosis by aggravating pre-existing heart failure [5]. It is now well established that uremic patients have a high prevalence of hyperhomocysteinemia. An interaction between hyperhomocysteinemia, hyperfibrinogenemia, and lipoprotein (a) excess has been thought to promote atherothrombosis and may contribute to the high incidence of CVD in maintenance dialysis patients [6].

The aim of this cross-sectional study was to analyze plasma sulfur amino acids (sAA) in hemodialysis (HD) patients with normal nutritional status, malnourished HD patients, and healthy subjects and to assess, in the HD patients, the relationship between tHcy levels, nutritional status, and CVD. The HD patients were then followed over a four-year period to assess the overall mortality in relationship to basal tHcy levels. A preliminary report of our findings that chronic renal failure patients with cardiovascular disease (CVD) had a lower tHcy level than patients without CVD was presented at the Annual Meeting of the American Society of Nephrology (abstract; Suliman et al, *J Am Soc Nephrol* 7:1325, 1996).

METHODS

Subjects

It was our intention that all HD patients at dialysis centers affiliated with Huddinge University Hospital should participate in a cross-sectional study of nutritional status, as previously reported [7]. Of the 128 patients who agreed to participate, plasma samples for determinations of Hcy and other related amino acids were obtained in 117 patients (71 male and 46 female) aged 65 (26 to 85) years. The causes of renal failure in the 117 patients included diabetic nephropathy ($N = 23$), chronic glomerulonephritis ($N = 34$), polycystic kidney disease ($N = 11$), pyelonephritis and interstitial nephritis ($N = 12$), and other unspecified diseases or unknown causes ($N = 37$). Thirteen of the diabetic patients were insulin dependent. Fifty-seven patients had ischemic CVD (ICVD) at the time of entering the study; that is, they had had one or more myocardial infarctions ($N = 18$), angina pectoris ($N = 14$), aortic aneurysm ($N = 1$), peripheral vascular diseases ($N = 18$), or a history of cerebrovascular accident ($N = 6$) with neurological symptoms following one or more attacks of strokes. All patients underwent electrocardiography (ECG). Thirteen patients had chronic heart failure but did not show clinical manifestations of ICVD or ischemic ECG changes. Eighty-six patients had no residual renal function, defined as a renal urea clearance <0.5 mL/min. The protein intake was estimated by calculating the protein equivalent of nitrogen appearance normalized to body weight (nPNA), based on urea kinetic modeling [8].

The patients were treated with oral sodium bicarbonate and calcium carbonate as required to prevent acidosis and hyperphosphatemia. Forty patients were treated with antihypertensive drugs such as angiotensin-converting enzyme inhibitors, β blockers, and calcium blockers. Fifteen patients with residual renal function were treated with high doses of furosemide (250 to 500 mg/day). Recombinant human erythropoietin was given to 66 patients. All patients were given routine supplements of water-soluble vitamins, including L-pyridoxine chloride (10 mg daily) but not folic acid or vitamin B_{12}.

Thirty-five healthy subjects (23 male and 12 female), aged 38 (21 to 64) years, were subjected to comparative analysis of nutritional parameters and amino acids, including tHcy.

The Ethics Committee of the Karolinska Institute at Huddinge University Hospital (Stockholm, Sweden) approved the protocol of the study, and informed consent was obtained from each HD patient and healthy subject.

Subjective global nutritional assessment

Subjective global nutritional assessment (SGNA) was used to evaluate the overall protein-energy nutritional status [9–11]. SGNA includes six subjective assessments, three based on the patient's history of weight loss, incidence of anorexia, and incidence of vomiting, and three based on the physician's grading of muscle wasting, presence of edema, and loss of subcutaneous fat. These variables were graded as: 1 = none, 2 = mild, 3 = moderate, and 4 = severe. The sum of the respective scores of the six subjective assessments was considered to be an ordinal variable in the statistical analysis. We divided the patients into two groups according to the SGNA score. Patients with an ordinal SGNA score between six and eight were placed in group I (normal nutritional status), and those with an ordinal SGNA score equal to or more than nine were placed in group II (mild to severe malnutrition).

Anthropometric measurements

Anthropometric and dynamometer measurements were carried out in the morning after the collection of blood samples. Triceps skinfold thickness (TSF) was measured in all subjects using the Harpenden caliper on the non-dominant arm in the healthy subjects and the fistula-free arm in the HD patients. The midarm muscle circumference (MAMC) was derived from the TSF and midarm circumference (MAC) as follows: MAMC = MAC − (π * TSF). Hand-grip strength (HGS) was measured using the Harpenden dynamometer. Subjects were instructed to apply as much hand-grip pressure as possible, using both hands. Dynamometry was repeated three times, and the highest score was recorded. The desirable body weight was calculated in accordance with the patient's height, sex, and frame-size match, using Metropolitan height and weight tables [12]. The individual values for anthropometric variables (TSF, MAMC, and HGS) were normalized by converting them to a percentage of the mean values of healthy subjects for the same sex. The SGNA and the anthropometric measurements were made by one investigator (A.R.Q.) who had experience with these methods. He was not aware of the biochemical data at the time of examination.

Table 1. Clinical, anthropometric and biochemical data (fasting, dialysis-free day) in hemodialysis patients

	Group I $N = 52$	Group II $N = 65$
Sex, male/female	37/15	34/31
Age, years (*median and range*)	56 (26–79)	66 (33–85)[c]
Diabetes, N (%)	5 (10%)	18 (28%)
CVD, N (%)	19 (36%)	51 (78%)
Body mass index *kg/m²*	23.8 ± 4.1	21.1 ± 3.7
% Desirable body weight[a]	105 ± 16	90 ± 14[c]
% Triceps skinfold thickness[b]	115 ± 51	73 ± 37[c]
% Mid-arm muscle circumference[b]	100 ± 9	90 ± 11[c]
% Hand-grip strength[b]	71 ± 27	47 ± 20[c]
Serum albumin *g/L*	35 ± 3	32 ± 5[c]
Serum creatinine *µmol/L*	739 ± 188	628 ± 211
Serum urea *mmol/L*	18.2 ± 4.9	19.7 ± 6.9
Standard bicarbonate *mmol/L*	24 ± 2	24 ± 3
Serum C-reactive protein *mg/L*	19 ± 19	24 ± 23
Plasma IGF-1 *ng/mL*	204 ± 97	147 ± 93[c]
Plasma folate *nmol/L*	15.5 ± 4.9	17.8 ± 6.8
Plasma B₁₂ *pmol/L*	416 ± 141	426 ± 161
Serum cholesterol *mmol/L*	5.7 ± 1.3	5.4 ± 1.5
Serum triglycerides *mmol/L*	1.7 ± 0.8	1.4 ± 0.6
nPNA *g/kg/day*	1.13 ± 0.23	1.07 ± 0.23

Data are mean ± SD. Abbreviations are: CVD, cardiovascular disease; nPNA, normalized protein equivalent of nitrogen appearance.

[a]The assessment % desirable body weight was based on medium frame-size individuals [12]

[b]Expressed as % of values in the HS of the same sex

[c]Significant differences between groups I and II, $P < 0.05$

Table 2. Plasma sulfur amino acid and plasma serine concentrations (µmol/L; mean ± SD) in 117 hemodialysis patients, divided into two groups as in Table 1, and 35 healthy subjects (HS)

	HS $N = 35$	Group I $N = 52$	Group II $N = 65$
Methionine	33.1 ± 9.9[a]	23.4 ± 5.3	20.7 ± 6.1[c]
Homocysteine (total)	8.9 ± 1.9[a]	31.2 ± 15.3	24.1 ± 11.6[c]
Cysteine (total)	315.2 ± 58.0[a]	433.2 ± 115.1	376.8 ± 125.5[b]
Taurine	49.6 ± 13.5[a]	41.2 ± 18.4	41.5 ± 20.1
Serine	122.3 ± 28.8[a]	83.2 ± 17.6	85.6 ± 21.3

Significant differences between all patients and controls are [a]$P < 0.001$, and between groups I and II, [b]$P < 0.05$ and [c]$P < 0.01$

Biochemical analyses

Venous blood samples from HD patients and control subjects were collected in the morning after an overnight fast. The HD patients were investigated on a midweek, dialysis-free day. As previously described, plasma-free amino acid concentrations [13] and tHcy and plasma total cysteine (tCys) concentrations [14] were determined with high-performance liquid chromatography. Serum albumin (S_{Alb}), serum creatinine (S_{Cr}), blood urea, serum C-reactive protein (S_{CRP}), and plasma insulin-like growth factor-1 (IGF-1) were determined as prescribed earlier [7]. Plasma folate concentration was determined with the Dualcount SPNB (solid phase no boil) radioimmunoassay kit from Diagnostic Product Corporation (Los Angeles, CA, USA).

Statistical analyses

Differences between the three groups were analyzed by analysis of variance (ANOVA). When the ANOVA was found to be significant at $P < 0.05$, Dunnett's test was used to compare the differences between the healthy subjects and HD patient groups. Comparisons between two groups were assessed for continuous variables by the Student's *t*-test, the Mann–Whitney test was used when distribution was skewed, and for nominal variables by the chi-square test. Spearman's rank correlation was used to determine correlations between variables. Stepwise multiple-regression analysis was applied to identify variables that independently predicted tHcy levels. Survival analysis was carried out using the Kaplan–Meier method. The data were censored for renal transplantation. All values are expressed as mean ± SD, unless otherwise indicated.

RESULTS

Fifty-two HD patients (44%) had a normal nutritional status (group I), and 65 patients (56%) were mildly to severely malnourished (group II), based on SGNA (Table 1). The distribution of primary causes of renal failure was essentially similar in the two groups of HD patients, except for a higher frequency of diabetes mellitus in group II ($N = 18$) than in group I ($N = 5$). CVD was present in 60% of all the patients and in 78% of the malnourished patients. The duration of HD treatment was significantly ($P = 0.03$) longer in group I, 25 months (range, 0.5 to 317 months) versus 14 months (range, 0.5 to 88 months) in group II. As expected, the malnourished patients were older and had a lower percentage of desirable body weight, MAC, TSF, HGS, S_{Alb}, and plasma IGF-1 (Table 1) than the well-nourished patients. The mean values of nPNA, plasma folate, and plasma vitamin B₁₂ concentrations were similar in the two patient groups.

Table 2 shows that the tHcy and tCys were significantly higher in the patient groups than in healthy subjects, whereas plasma methionine (Met), plasma taurine (Tau), and plasma serine (Ser) concentrations were lower in the HD patients. The Met, tHcy, and tCys concentrations were significantly lower in group II (malnourished) patients than in group I. There were no significant differences in Tau and Ser concentrations between the two patient groups.

Hyperhomocysteinemia was present in the vast majority of the 117 HD patients, 95% of all patients, 90% of the HD patients with CVD, and 96% of the patients without CVD had tHcy levels >13 µmol/L (>95th percentile of the tHcy level in healthy subjects). Dividing the HD patients into two groups, based on presence of

Table 3. Significance of differences between hemodialysis patients with or without cardiovascular diseases

	No CVD	CVD	P
Male/female	33/14	38/32	
Age *years*	56 ± 16	64 ± 12	0.0025[a]
Prevalence of malnutrition	30%	70%	0.0001[b]
Total plasma homocysteine *μmol/L (all patients)*	31 ± 15	24 ± 12	0.016[a]
Total plasma homocysteine *μmol/L (ICVD, N = 57)*[d]		23 ± 10	0.003
Total plasma cysteine, *μmol/L*	411 ± 128	395 ± 120	NS[a]
Plasma IGF-1 *ng/mL*	200 ± 111	158 ± 89	0.03[c]
Serum albumin *g/L (all patients)*	34.5 ± 4.8	32.5 ± 4.5	0.01[a]
Serum albumin *g/L (ICVD, N = 57)*[d]		32.7 ± 4.1	0.04[a]
Blood hemoglobin *g/L*	103 ± 15	96 ± 15	0.01[a]
Serum creatinine *μmol/L*	756 ± 211	635 ± 192	0.007[a]
Prevalence of serum C-reactive protein >10 mg/L	51%	47%	NS[b]
Plasma folate *nmol/L*	16.7 ± 6.1	17.1 ± 6.4	NS[a]
Plasma B$_{12}$ *pmol/L*	422 ± 155	423 ± 153	NS[a]

[a]Significance was tested with Student's *t*-test
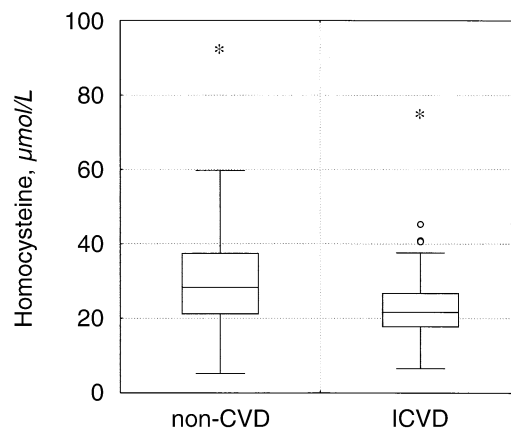[b]Significance was tested with chi-square test
[c]Significance was tested with the nonparametric Mann–Whitney test
[d]Patients with chronic cardiac failure without signs of ischemic cardiovascular disease (ICVD) were excluded

Fig. 1. Total plasma homocysteine concentrations in hemodialysis (HD) patients with ischemic cardiovascular disease (ICVD, N = 57) and in HD patients without cardiovascular disease (non-CVD, N = 47). Thirteen patients who had chronic heart failure without signs of ICVD were excluded from the analysis. Symbols are: (whiskers) maximum and minimum non-outliers; (□) median, 75% and 25%; (°) outliers; (*) extremes. Difference between the groups: *P* = 0.003.

CVD, as shown in Table 3, we found that HD patients with CVD were older and had lower S$_{Alb}$ levels than those without CVD. The tHcy concentration was lower in HD patients with CVD than in those without CVD (Table 3), and this difference was also present after excluding the 13 HD patients with chronic heart failure who had no signs of ischemia (Table 3 and Fig. 1). The plasma folate and vitamin B$_{12}$ concentrations were similar in the two patient groups, and the concentrations of folate and B$_{12}$, in all HD patients, were within the normal range [15]. The levels of plasma IGF-1, blood hemoglobin, and S$_{Cr}$ were significantly lower in patients with CVD. The proportion of patients with serum S$_{CRP}$ >10 mg/L was also similar in the two groups of patients.

There were more males among the HD patients with CVD than in those without CVD (72 vs. 52%, *P* = 0.03). Therefore, we also evaluated males and females separately to determine the tHcy concentration. In male HD patients, the tHcy level was significantly lower in patients with CVD than in those without (24 ± 13 vs. 32 ± 16 μmol/L, respectively, *P* = 0.02), whereas the levels were not significantly different in female HD patients with or without CVD (25 ± 12 vs. 28 ± 11 μmol/L, respectively, *P* = 0.5). Among the 52 male patients aged 50 years or more at the time of the study, the tHcy concentration in those with CVD (*N* = 32) was 24.9 ± 14.2 μmol/L, and in those without CVD (*N* = 20), it was 31.6 ± 18.2 μmol/L (*P* = 0.07).

The median age of the HD patients was 65 years. Those more than 65 years of age had lower S$_{Alb}$ levels

than the HD patients less than 65 years old (32 ± 4 vs. 35 ± 5 μmol/L, *P* < 0.001) and lower tHcy (24.5 ± 8.6 vs. 28.9 ± 15.3 μmol/L, *P* = 0.05), as compared with the younger patients.

A correlation matrix, presenting Spearman's rank correlation coefficients for the HD patients, is shown in Table 4. The SGNA score was negatively correlated with S$_{Alb}$, S$_{Cr}$, tCys, tHcy, IGF-1, and Met levels, but it was not significantly correlated with nPNA and S$_{CRP}$. Plasma tHcy was positively correlated with S$_{Alb}$, tCys, and S$_{Cr}$ levels, as shown in Figure 2, and was also correlated with Met, but did not correlate with S$_{CRP}$ (Table 4), plasma folate (*r* = −0.15, *P* = 0.11), and plasma B$_{12}$ (*r* = −0.05, *P* = 0.6). Serum albumin was also positively correlated with Met, tCys, and IGF-1, and negatively correlated with S$_{CRP}$. In addition, tCys was positively correlated with Met and S$_{Cr}$. Normalized PNA was positively correlated with Met and inversely with S$_{CRP}$.

Stepwise multiple-regression analysis was used to find significant independent predictors of tHcy in the HD patients (Table 5). Plasma Cys (r^2 = 0.23) was the strongest predictor, followed by S$_{Alb}$ (r^2 = 0.11) and protein intake (r^2 = 0.04), as assessed by nPNA. The regression model could explain 38% of the variation in tHcy. Omitting tCys from the model reduced the adjusted total r^2 from 0.38 to 0.17.

The median level of tHcy in the HD patients was 24 μmol/L. The cumulative Kaplan–Meier survival analysis of patients with tHcy ≥24 μmol/L and <24 μmol/L showed a significantly worse survival in those with lower tHcy levels (Fig. 3). Figure 3 represents the overall mortality of HD patients and not only mortality caused by cardiovascular events.

Table 4. Spearman rank correlation matrix for eight variables

Variable	SGNA score	S_{CRP}	p-IGF-1	nPNA	S_{Alb}	S_{Cr}	p-methionine	p-Hcy
S_{CRP}	0.17							
p-IGF-1	−0.27[b]	0.27[b]						
nPNA	−0.13	−0.20[a]	0.33[c]					
S_{Alb}	−0.42[c]	−0.23[a]	0.42[c]	0.23[a]				
S_{Cr}	−0.32[c]	−0.30[c]	0.37[c]	0.09	0.13			
p-methionine	−0.21[a]	−0.13	0.11	0.22[a]	0.31[c]	0.05		
p-Hcy	−0.28[b]	−0.18	0.07	0.12	0.39[c]	0.30[c]	0.27[b]	
p-Cys	−0.29[b]	−0.16	0.04	0.17	0.26[b]	0.36[c]	0.24[a]	0.60[c]

Abbreviations are in the **Appendix**.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$
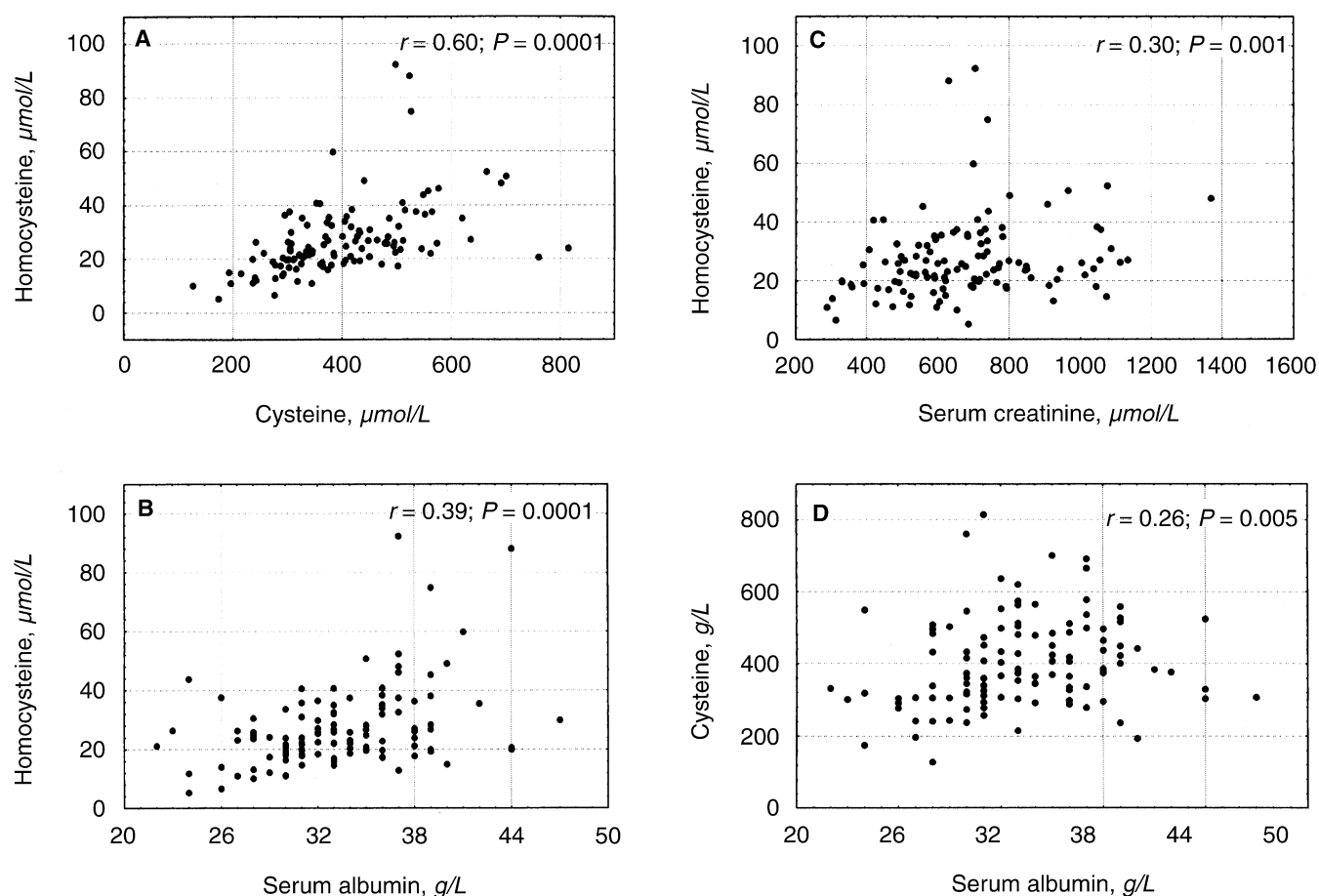
Fig. 2. Relationship between total plasma homocysteine and total plasma cysteine (*A*), serum albumin (*B*), and serum creatinine (*C*), and the relationship between total plasma cysteine and serum albumin (*D*) in 117 hemodialysis patients.

DISCUSSION

The results of the present study demonstrate a high prevalence of hyperhomocysteinemia in HD patients, thus confirming earlier reports. However, the precise mechanism of hyperhomocysteinemia in chronic renal failure remains unclear. Reduced renal excretion [16] and decreased renal uptake [17, 18] of Hcy have been reported, but recent findings do not show that renal elimination of Hcy is of any significance in normal hu-

mans [19]. Disturbed methylation reactions caused by uremia or cofactor (folate, vitamin B_6) deficiency have also been thought to contribute to hyperhomocysteinemia [20]. Moreover, the extrarenal Hcy metabolism may decrease because of the inhibition by retained metabolites [21]. Supplementation with vitamins (folate, vitamin B_6) reduces but generally fails to normalize elevated Hcy levels in uremia [22].

We observed (Table 2) that the levels of tHcy and

Table 5. Multivariate analysis of predictors of plasma homocysteine in 117 HD patients

|	Estimate	Standard error	r^2	P
Intercept	−26.33	7.9		0.0012
Plasma cysteine $\mu mol/L$	0.05	0.008	0.23	<0.0001
Serum albumin g/L	1.02	0.23	0.34	<0.0001
nPNA groups (<1.1 vs. ≥1.1)	2.98	1.05	0.38	0.006
CVD vs. others	−1.99	1.05	0.40	0.06

Adjusted total $r^2 = 0.38$.

tCys were elevated and significantly correlated (Fig. 2), and that in multifactorial analysis tCys was the strongest predictor of tHcy. Moreover, the levels of Met (the precursor essential sAA) and Tau (an end-product of Met metabolism) were low. These findings are consistent with our previous observation in smaller groups of HD patients [13, 22, 23]. We also reported earlier that plasma and muscle Tau are depleted in dialysis patients [24–26] and that the plasma levels of cysteinesulfinic acid (CSA) are elevated [13]. We speculated earlier that inhibition of Tau synthesis from CSA might have a role in the development of hyperhomocysteinemia in renal failure patients [13]. However, a recent study using stable isotopes demonstrates that homocysteine remethylation rather than transsulfuration is decreased in renal failure patients [27].

Homocysteine, nutritional status, and serum albumin

To investigate the relationship between tHcy levels and nutritional status in HD patients, we divided the patients according to the SGNA into two groups. The patients in group II (malnourished group; Table 1) had low S_{Alb} and anthropometric measurements (percentage of desirable body weight, fat mass, MAMC, TSF, and HGS), as compared with the patients in group I (well-nourished group). The results of the present study show also that tHcy levels are consistently elevated in the HD patients (95%), and this change is more marked in well-nourished patients, whereas malnourished patients had lower tHcy concentrations (Table 2). Thus, one factor that may have directly contributed to the lower tHcy levels in the malnourished HD patients is the lower levels of S_{Alb}. In the general population, protein-bound Hcy accounts for more than 65% of total Hcy [23, 28, 29] and the main Hcy carrier in plasma is S_{Alb} [30]. In uremic patients, the fraction of protein-bound Hcy is similar to that in normal subjects; however, it is higher in dialyzed than in nondialyzed uremic patients [23]. Hence, it seems likely that S_{Alb} is a strong determinant of tHcy in HD patients, and this may contribute to the low tHcy levels in malnourished patients.

Serum albumin is considered a key index of nutritional status, and it is commonly used to assess protein malnu-
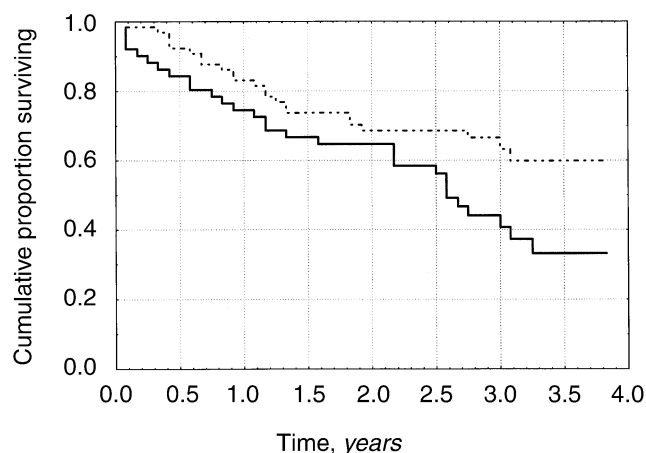
Fig. 3. Kaplan–Meier plots of the survival rate of hemodialysis patients. The patients were divided into two groups according to the plasma total homocysteine level, that is, <24 μmol/L (solid line) and ≥24 μmol/L (dashed line). This figure represents the overall mortality of HD patients and not only mortality caused by cardiovascular events. Difference between the groups: $P = 0.02$.

trition, based on the concept that the level of S_{Alb} reflects the visceral protein status. However, protein intake and various catabolic factors, as well as nonnutritional factors may influence S_{Alb} [31]. Accordingly, S_{Alb} levels may not only reflect protein malnutrition [32, 33]. In this study, S_{Alb} was related to inflammation, as assessed by the S_{CRP} (inversely correlated), but no relationship was found between S_{CRP} and tHcy (Table 4), therefore suggesting that the relationship between tHcy and S_{Alb} most likely was not due to inflammation.

In this study, the protein intake, estimated from the nPNA, did not differ between the two groups of patients. However, in a stepwise multiple-regression model, we found that nPNA, an indicator of protein intake, was indeed a predictor of the tHcy level (Table 5). Moreover, the correlations between Met and tHcy, as well as between Met and nPNA (Table 4), may indicate that the tHcy level in HD patients was dependent on the intake of dietary protein, which is the only source of Met. Inadequate concentrations of the vitamin cofactors (folate, vitamin B_{12} and vitamin B_6) are contributing factors for hyperhomocysteinemia. The plasma concentrations of folate and B_{12} were within the normal range in our patients and were similar in the well-nourished and malnourished patients. We did not measure vitamin B_6, but our patients were routinely supplemented with pyridoxine.

Age is a determinant of both the tHcy and tCys levels, and this physiological age-related increase may be linked to the age-related decrease in renal function [34]. However, these levels were in fact lower in our older patients, possibly because they also had a lower S_{Alb} than the younger patients, considering that tHcy and tCys are correlated to S_{Alb} (Table 4).

Homocysteine and cardiovascular disease

In the present study, the tHcy levels in HD patients were three to four times higher than in the controls, with only 5% of the patients having normal tHcy levels, hyperhomocysteinemia being defined as >95th percentile of the normal range. Assuming that hyperhomocysteinemia carries an increased risk of ICVD, this implies that practically all chronic HD patients are exposed to this risk. This is in accordance with the high prevalence of CVD in the patients in this study and in several previous studies. On the other hand, our patients with ICVD had significantly lower (although still elevated) tHcy levels than those without CVD, a finding that may seem paradoxical if one assumes that hyperhomocysteinemia, in particular absolute tHcy levels, carry more risk for CVD. However, these observations do not exclude a role for hyperhomocysteinemia, since almost all patients would be exposed to this risk and since other factors might be present that confound the relationship between tHcy levels and CVD. An interaction between hyperhomocysteinemia, hyperfibrinogenemia, and lipoprotein(a) excess has been suggested [6]. It is noteworthy that men with CVD had significantly lower tHcy levels, but we found no difference in tHcy levels between females with or without CVD.

Conflicting findings have been reported regarding the association between the tHcy level and the prevalence of CVD in chronic renal failure patients. In the study by Robinson et al in HD patients, risk factor analyses showed a significant relationship between the tHcy level and atherosclerotic CVD, even after adjustment for other risk factors [35]. Others have reported a similar association [36–38]. Moreover, three prospective studies demonstrated that a high tHcy level indeed was related to cardiovascular events in chronic renal failure patients [39–41]. In contrast, Bostom et al found no relationship between the fasting tHcy level and prevalence of CVD in dialyzed uremic patients, using crude or multiple logistic regression analyses adjusted for other risk factors [42]. In addition, Klusmann et al (abstract; *Wien Klin Wochenschr* 110:26, 1998) reported that there was no difference in tHcy levels between uremic patients with coronary artery disease and those without; however, they could not identify tHcy as an independent cardiovascular risk factor in their patients.

As shown in the present study, HD patients with ICVD have a higher prevalence of malnutrition and hypoalbuminemia and a lower protein intake than those without ICVD. Foley et al also reported a strong relationship between hypoalbuminemia and CVD in end-stage renal disease (ESRD) patients, and suggested that hypoalbuminemia is an important risk factor for CVD in these patients [43]. However, it is not clear how cardiac disease, hypoalbuminemia, and malnutrition in uremic patients are interrelated [5]. Hypoalbuminemia and malnutrition per se may be risk factors for cardiac disease, but cardiac disease may also lead to malnutrition, as has been demonstrated in the general population [44]. Moreover, a common mechanism for the development of CVD and malnutrition might be cytokine activation associated with reduced renal function or inflammation, since proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor) may suppress appetite, cause muscle proteolysis and hypoalbuminemia, and may be involved in the processes leading to atherosclerosis [5].

We observed that the HD patients with lower tHcy levels (<24 μmol/L) had a significantly worse survival rate than those with higher levels ($\geq$24 μmol/L; Fig. 3). This may seem paradoxical in view of the established relationship between elevated tHcy levels and increased mortality in the general population [45], as well as in uremic patients [39–41]. However, we are aware that tHcy levels are influenced by various factors such as nutritional status, S_{Alb}, and the presence of CVD, which are interrelated in a complex manner and also may influence the overall mortality. Unfortunately, it is not feasible to evaluate the independent effects of tHcy and these other factors on mortality because of the limited number of patients in the present study. Interestingly, a recent study by Sirrs et al also has shown that HD patients with higher tHcy levels appear to have a better survival rate than those with lower levels [46].

Since both lower tHcy levels and a higher prevalence of CVD were associated with hypoalbuminemia and malnutrition, this implies that nutritional status and the S_{Alb} concentration should be taken into consideration when evaluating tHcy as a risk factor. Currently, we do not know whether there is a critical plasma level at which Hcy becomes atherogenic in uremic patients or whether there is a relationship between atherogenic effects and absolute levels of Hcy above that critical level. Moreover, it is not known to what extent hyperhomocysteinemia in uremic patients acts in concert with other potential risk factors for CVD, such as hypoalbuminemia, malnutrition, lipid abnormalities, inflammation, hyperfibrinogenemia, and oxidative stress. Hence, the higher mortality in our patients with lower (although still high) tHcy may be related to the higher prevalence of malnutrition, lower protein intake, and lower S_{Alb} in the HD patients with CVD, considering that malnutrition is a risk factor for mortality and that CVD is the most common cause of death in ESRD patients.

In summary, our study indicates that tCys, nutritional status, and protein intake may influence the tHcy levels in HD patients. A higher absolute level of plasma tHcy in HD patients appeared to be associated with a lower prevalence of CVD and better survival, possibly related to increased protein intake, better nutritional status, and a higher S_{Alb} level. This apparently paradoxical relation-

ship, however, does not contradict that hyperhomocysteinemia, present in 95% of the HD patients, is a risk factor for CVD in uremia.

APPENDIX

Abbreviations used in this article are: CSA, cysteinesulfinic acid; CVD, cardiovascular disease; ECG, electrocardiography; ESRD, end-stage renal disease; HD, hemodialysis; HGS, hand-grip strength; ICVD, ischemic cardiovascular disease; MAC, mid-arm circumference; MAMC, mid-arm muscle circumference; Met, methionine; nPNA, normalized protein equivalent of nitrogen appearance; sAA, sulfur amino acids; S_{Alb}, serum albumin; Ser, serine; SGNA, subjective global nutritional assessment; Tau, taurine; tCys, total plasma cysteine; tHcy, total plasma homocysteine; TSF, triceps skinfold thickness.

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